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BIOSYNTHESIS OF YEAST CAROTENOIDS

KENNETH L. SIMPSON, T. O. M. NAKAYAMA, AND C. O. CHICHESTER

Department of Food Science and Technology, University of California, Davis, California

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ABSTRACT

SIMPSON, KENNETH L. (University of California, Davis), T. O. M. NAKAYAMA, AND C. O. CHICHESTER. Biosynthesis of yeast carotenoids. J. Bacteriol. 88:1688-1694. 1964.—The biosynthesis of carotenoids was followed in *Rhodotorula glutinis* and in a new strain, 62-506. The treatment of the growing cultures by methylheptenone, or ionone, vapors permitted observations of the intermediates in the biosynthetic pathway. On the basis of concentration changes and accumulation in blocked pathways, the sequence of carotenoid formation is postulated as phytoene, phytofluene, ζ-carotene, neurosporene, β-zeacarotene, γ-carotene, torulin, a C_{40} aldehyde, and torularhodin. Torulin and torularhodin were established as the main carotenoids of 62-506.

Unified concepts for the biosynthesis of the carotenoids have been recently proposed by Jensen, Cohen-Bazire, and Stanier (1961) and Porter and Anderson (1962). The conclusions drawn from earlier studies of yeast carotenoids, however, conflict in part with the transformations of carotenoids believed to take place in other carotenoid-forming systems. The yeast studies have been confined mainly to mutants of *Rhodotorula*.

Bonner et al. (1946) studied seven mutants (produced by treating the normal strain with ultraviolet radiation) isolated from *R. rubra* whose color ranged from red to colorless. Torulin, γ- and β-carotene, and phytofluene, along with pigments "A" and "B," were isolated. On the basis of this study, the authors proposed a parallel pathway for the formation of torulin and the yellow carotenoids, with phytofluene as a common precursor.

Villoutreix (1960) concluded, after investigating the carotenoids isolated from the mutants of *R. mucilaginosa* (produced by treating the normal strain with ultraviolet radiation), that the carotenoids of yeast are not mutually related. Of the seven mutants studied, five contained torularhodin; one contained spirilloxanthin; one, lycopene; four, torulin; five, neurosporene; six, γ-carotene, five, ζ-carotene; two, pigment X; six, β-carotene; and three, phytofluene.

Kayser and Villoutreix (1961) later examined the carotenoids of a mutant of *R. mucilaginosa* (produced by irradiating the normal strain with X rays), and proposed the pigments X and β-carotene as the precursors of both torularhodin and torulin.

Nakayama, Mackinney, and Phaff (1954) investigated the effect of temperature on the formation of the carotenoids of *R. glutinis*. At 5 C, β- and γ-carotene were the main pigments formed, whereas at 25 C larger amounts of torulin and torularhodin were formed, accompanied by a decrease in γ- and β-carotene.

We report on the biosynthesis of the carotenoids in *R. glutinis*. The changes in carotenoid synthesis were brought about by culturing at temperatures of 5 and 25 C, and by treatment with the vapors of methylheptenone and β-ionone. The carotenoids of a new isolate were determined to be primarily γ-carotene, torulin, and torularhodin.

MATERIALS AND METHODS

Some of the characteristics of *R. glutinis* (Fres.) Harrison 48-23T have been described previously (Nakayama et al., 1954; Simpson, Nakayama, and Chichester, 1964). The very lightly pigmented yeast 62-506 was isolated from trout stomach, and appears to be a new species of *Rhodotorula* (H. J. Phaff and J. F. T. Spencer, *personal communication*).

Petroleum ether (boiling range, 30 to 60 C; analytical reagent grade) was distilled prior to use, and was further purified by percolating the distilled product over silica gel (Graff, O'Connor, and Skan, 1944). Spectral grade petroleum ether was used for chromatographic separation of phytoene. Anhydrous ethyl ether (analytical reagent grade) was freed from peroxide by passage through alumina before use. All other solvents of analytical reagent grade were used as received.

Hyflo Super-Cel was obtained from the Johns-Manville Corp., New York, N.Y. Magnesium oxide was obtained from the Baker Chemical Co., North Phillipsburg, N.J. Cellulose ashless powder, standard grade (Whatman), was obtained from Van Waters and Rodgers, San Francisco, Calif., W. & R. Balston Ltd. Alumina, neutral activity grade I, was obtained from Alupharm Chemicals, New Orleans, La. Methylheptenone and β-ionone were obtained from Fritzsche Bros. Inc., New York, N.Y.

R. glutinis 48-23T was cultivated on a medium consisting of 10% (v/v) yeast autolysate, 2.5% agar, and 5% glucose (Nakayama et al., 1954). A 100-ml amount of medium was autoclaved in shasta flasks (flat whiskey bottles of 473-ml capacity) and allowed to solidify with the flasks lying on one side. This provided an agar surface of approximately 100 cm^2 for growth. In some experiments both strains 48-23T and 62-506 were cultivated in 250-ml Erlenmeyer flasks containing 50 ml of a liquid medium consisting of 0.5% yeast autolysate and 5% glucose broth. Broth cultures were incubated at 20 C on a rotary shaker. Cultures were grown in the presence of methylheptenone or β-ionone vapors by simply dipping a cork (diameter, 1.0 cm) into these liquids, and touching the cork surface lightly to the tip of the cotton plugs, which were then reinserted into the mouths of the culture flasks.

The yeast cells were harvested, washed, and then disrupted in a modified French press (Simpson et al., 1963, 1964). The pigments were extracted with acetone, transferred to petroleum ether, and chromatographed on magnesium oxide-Hyflo Super-Cel (1:2, w/w) as described previously (Simpson et al., 1964). Torularhodin was adsorbed on the column under these conditions, while the other pigments were eluted by the developing solvents (petroleum ether, ethyl ether, and methyl alcohol).

Potassium hydroxide-methyl alcohol solution (200 ml; 10%, w/v) was added to the solution containing the crude mixture of pigments eluted from the column. Saponification was completed by heating for 5 min on a steam table. Petroleum ether was added to the mixture, which was washed free from water-soluble materials. The petroleum ether solution was then dried over anhydrous sodium sulfate.

The pigments were rechromatographed on magnesium oxide-Super-Cel with petroleum ether. "Crude" phytoene and phytofluene were eluted from the column, and the other bands were cut from the columns and individually eluted with solvents. The order of adsorption was β-carotene, β-zeacarotene, ζ-carotene, γ-carotene, neurosporene, and torulin. All of these, except torulin and torularhodin, were eluted from the column material with acetone. They were transferred to petroleum ether, and washed free from acetone with water washes.

Torulin was eluted with ethyl ether and methyl alcohol, and torularhodin was eluted with acetic acid-ethyl ether (1:10). The water-soluble compounds were removed by water washes, and the ethyl ether was dried over anhydrous sodium sulfate. Torularhodin and torulin were taken to dryness in a rotary flash evaporator, and then were dissolved in $CHCl_3$ and petroleum ether, respectively.

Torulin was rechromatographed on cellulose, where it separated into an orange and a red band. The red band exhibited absorption maxima corresponding to published values for torulin. Iodine catalysis of torulin produced a pigment whose light-absorption curve closely resembled that of the orange product. This suggested that the orange torulin was an isomeric (*trans* → *cis*) conversion product probably produced in the isolation and purification procedures (Simpson et al., 1963).

Neurosporene, phytofluene, and β-carotene were rechromatographed on magnesium oxide-Super-Cel with petroleum ether as the developing agent. ζ-Carotene was rechromatographed on alumina grade II. β-Zeacarotene, included in the ζ-carotene band, was eluted just before ζ-carotene with 15% ethyl ether-85% petroleum ether. The combined β-zeacarotene fractions were rechromatographed on alumina grade I in petroleum ether. β-Carotene was eluted with 25% ethyl ether, and β-zeacarotene and β_1-zeacarotene were eluted with 40% ethyl ether.

γ-Carotene was rechromatographed on alumina grade I with 50% ethyl ether as the eluting solvent.

Phytofluene was rechromatographed on alumina grade I. Ethyl ether (1%) in petroleum ether was used as a developing solvent, and was added after 60 ml of petroleum ether had passed through the column.

The carotenoids were identified by their positions on the columns, and by their light-absorp-

tion curves. Close agreement was obtained between the absorption maxima of the isolated pigments and published data. Table 1 lists the characteristic wavelength maxima and the extinction values used for quantitative determination.

RESULTS

R. glutinis 48-23T, which altered the composition of its carotenoids when cultured at 5 C, was reinvestigated. While the major pigments had been identified by Nakayama et al. (1954), it was felt that minor pigments might have been lost in the procedures utilized in the original investigation.

A summary of the differences in carotenoid concentration in cultures grown at 5 and 25 C is shown in Table 2. The effect of temperature was twofold. (i) The amount of cell material per culture was less at the lower temperature, and the concentration (micrograms per gram, dry weight) of carotenoids isolated under both conditions was similar. (ii) The concentration (micrograms per gram, dry weight) of isolable neurosporene and γ-carotene was relatively unchanged, and β-zeacarotene was moderately changed by growth at the lower temperatures. The most dramatic change was observed in the levels of β-carotene, torulin, and torularhodin.

These data can be explained in two ways. The red pigments could be formed sequentially from β-carotene, and at low temperature the system is blocked at this point. A more likely proposal, however, based on known structures of torulin (Rüegg et al., 1961*a*, *b*) and torularhodin (Isler et al., 1959), would involve a monocyclic carotenoid, such as γ-carotene, as a common precursor.

Mackinney et al. (1952) found that β-ionone vapor stimulated to a marked degree the amount of carotenoids, particularly β-carotene, formed by *Phycomyces blakesleeanus*. Reyes (1963) showed that sterol, as well as carotenoid formation, was stimulated by β-ionone. The effect was postulated as one of an inhibition of a negative feedback mechanism acting at the pathway level of the phosphorylated derivatives of mevalonic acid.

Methylheptenone vapor has been shown (Nakayama et al., 1957) to stimulate the formation of phytoene, phytofluene, ζ-carotene, neurosporene, and to a lesser extent β-carotene in *Phycomyces*.

Both β-ionone and methylheptenone stimulated the formation of phytoene, phytofluene, ζ-carotene, neurosporene, and β-zeacarotene in *R. glutinis* (Table 3). The formation of β-carotene, torulin, and torularhodin, however, was greatly suppressed by these agents. γ-Carotene concentration did not change with the methylheptenone treatment, but decreased when the culture was treated with β-ionone. The effect of β-ionone on β-carotene synthesis was to decrease its concentration; a more dramatic effect was demonstrable by adding the β-ionone at the time of inoculation. Under these conditions only 5.1 μg/g of β-carotene and 9.3 μg/g of γ-carotene were formed.

The overall effect of the administration of

TABLE 1. *Comparison of absorption maxima of pigments isolated from Rhodotorula glutinis with literature values*

Carotenoid	Wavelength maxima* (mμ)		$E_{1\ cm}^{1\%}$ literature value	Literature citation
	Literature value	Experimental value		
Phytoene	285	285	850	Rabourn and Quackenbush, 1953
Phytofluene	348	348	1,540	Koe and Zechmeister, 1952
ζ-Carotene	399	400	2,500	Nash, Quackenbush, and Porter, 1948
Neurosporene	438	439	2,740	Nakayama, 1958
β-Zeacarotene	426	426	1,940	Petzold, Quackenbush, and McQuistan, 1959
β_1-Zeacarotene	427	427	1,800	Petzold et al., 1959
γ-Carotene	460	460	2,760	Goodwin, 1956
β-Carotene	451	452	2,500	Karrer and Jucker, 1950
Torulin	484	485	2,680	Simpson, Nakayama, and Chichester, 1964
Torularhodin	515	515	1,932	Karrer and Jucker, 1950

* All determinations were made in petroleum ether, except torularhodin, for which $CHCl_3$ was used.

TABLE 2. *Concentrations of various carotenoids produced by Rhodotorula glutinis 48-23T after growth at 5 and 25 C*

Carotenoid	Concn of carotenoid			
	Incubated 12 days at 25 C*		Incubated 21 days at 5 C*	
	Amt	Per cent	Amt	Per cent
	μg/g†		μg/g†	
Phytoene	—	—	—	—
Phytofluene	—	—	Trace	—
ζ-Carotene	—	—	—	—
Neurosporene	3.4	1.3	4.5	2.2
β-Zeacarotene	2.3	0.9	10.8	5.2
γ-Carotene	32.3	12.5	24.2	11.7
β-Carotene	64.4	25.2	133.0	64.0
Torularhodin	62.0	24.3	9.9	4.8
Torulin	71.0	27.8	9.1	4.4
Total carotenoids.	235.0	100.0	207.0	100.0

* Cultures were grown on the solid medium in shasta flasks, and harvested as described in Materials and Methods.

† Per gram of cells on a dry weight basis. The average dry weight of cells per culture flasks at 25 C was 0.72 g, and at 5 C, 0.44 g.

β-ionone and methylheptenone was to decrease the total amount of carotenoids formed, and to slow culture growth.

The effect of β-ionone on β-carotene synthesis by *Rhodotorula* presents a striking departure from the pattern exhibited by *Phycomyces*.

A new yeast isolate 62-506 was examined for the presence of carotenoids. The culture was almost colorless on wort agar slants, although torulin, torularhodin, and γ-carotene were detected in preliminary assays. When cultured in shake flasks for 9 days, small amounts of neurosporene and β-carotene were detected in addition to the three main pigments.

Figure 1 shows the kinetics of the formation of three pigments in 62-506. γ-Carotene was formed early in the growth cycle, reached a maximal concentration, and then decreased. Simultaneous increases were noted in torulin and torularhodin. Although total pigment content decreased, the percentage of the latter pigments increased.

DISCUSSION

Bonner et al. (1946) reported the first general scheme for the transformation of the yeast carotenoids. Phytofluene, γ-carotene, β-carotene, and torulin were identified in that study. From the light-absorption values and their positions on the column, pigment "A" of Bonner et al. (1946)

TABLE 3. *Influence of methylheptenone and β-ionone vapors on concentrations of various carotenoids produced by Rhodotorula glutinis 48-23T*

Carotenoid	Concn of carotenoid					
	With methyl-heptenone vapor*		With β-ionone vapor*		No vapors*	
	Amt†	Per cent	Amt†	Per cent	Amt†	Per cent
	μg/g		μg/g		μg/g	
Phytoene	63.0	39.3	86.5	52.1	—	—
Phytofluene	8.5	5.3	5.1	3.1	Trace	—
ζ-Carotene	4.8	3.0	3.6	2.2	—	—
Neurosporene	15.5	9.6	6.4	3.8	Trace	—
β-Zeacarotene	13.8	8.6	14.7	8.7	3.6	1.4
γ-Carotene	15.2	9.5	2.2	1.3	26.7	10.4
β-Carotene	29.8	18.5	40.4	24.3	162.0	63.3
Torularhodin	5.8	3.6	5.9	3.5	38.6	9.6
Torulin	4.2	2.6	1.5	0.9	25.8	10.0
Total carotenoids	161.0	100.0	166.0	100.0	257.0	100.0

* Cultures were grown in the broth medium with shaking at 20 C, and harvested as described in Materials and Methods. The vapors were introduced after 2 days of growth.

† Per gram of cells on a dry weight basis. The average dry weight of cells per culture flask was 0.20 g (methylheptenone), 0.19 g (β-ionone), and 0.22 g (no vapors).

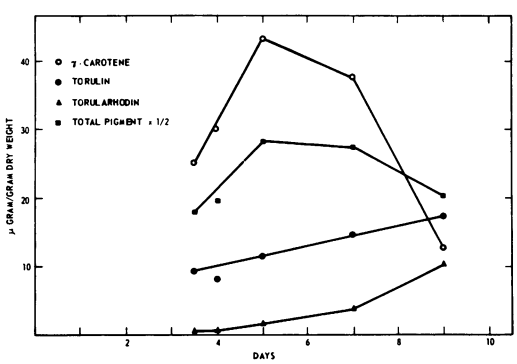

FIG. 1. *Changes in the individual concentration of the three major carotenoid pigments and the total pigments as a function of growth time in culture 62-506.*

was probably neurosporene, and pigment "B," ζ-carotene. The presence of torularhodin, phytoene, and β-zeacarotene was not indicated. At the time of their report, many of the carotenoid structures were unknown; thus, a relationship based on structure was then not possible.

Villoutreix (1960) isolated phytoene, phytofluene, ζ-carotene, neurosporene, pigment X (probably identical to β-zeacarotene), γ-carotene, torularhodin, torulin, and β-carotene. In addition, one mutant, which only formed acyclic polyenes, also formed lycopene and spirilloxanthin. Villoutreix (1960) concluded, on the basis of the analysis of the amounts of the various carotenoids isolated, that the various yeast polyenes were not mutually related.

In a subsequent report, Kayser and Villoutreix (1961) proposed, on the basis of the alternation of the carotenoids of an X-ray mutant of *R. mucilaginosa*, that pigment X and γ-carotene were the precursors of torulin and torularhodin.

In the present report, the biosynthesis of *Rhodotorula* carotenoids was studied by altering the carotenoid formation in 48-23T. By use of the temperature-sensitive properties of *R. glutinis*, γ-carotene would appear to be at a branch point in the biosynthetic pathway. A temperature-sensitive system subsequent to γ-carotene would thus seem to be responsible for the channeling of intermediates to either of the red carotenoids of β-carotene.

The change in the concentration relationships of the carotenoids in growing cultures of 62-506 reinforced the position of γ-carotene in the biosynthetic pathway. γ-Carotene does not constitute the major pigment in *Rhodotorula* grown at 25 C, but is normally found at concentrations approximating 25% of the total pigment (Nakayama et al., 1954). In 62-506, γ-carotene concentration approached 65% of the total carotenoid after 5 days of growth; therefore, it must represent a major product in the isoprenoid pathway. Its decrease with time, coupled with a simultaneous increase in structurally related products, could indicate an interconvertibility, even though the relation is not stoichiometric.

If the assumption is made that in the latter stages of growth a general loss of all pigments occurs (Fig. 1), and that all of the pigments have approximately the same relative stability, the increase in concentration of torularhodin with time is very nearly equal to the drop in γ-carotene concentration. The abrupt change in γ-carotene in any case is indicative of a major change in metabolism. The metabolic changes observed are similar to many previous observations (Jensen et al., 1961; Claes, 1954, 1956, 1958).

In *P. blakesleeanus* (Chichester, Wong, and Mackinney, 1954), it was shown that methylheptenone blocks carotenoid synthesis, largely at the phytoene level, although some accumulation of phytofluene was noted. It was postulated that the dehydrogenation steps in the pathway were blocked (Porter and Lincoln, 1950; Jensen et al., 1958), and thus the accumulation of the more saturated intermediates occurred. The same effect was noted when the cultures were treated with citral or cyclocitral (Chichester et al., 1954), whose structures in many respects are similar to that of methylheptenone and ionone. Treatment of carotenoid-producing systems with diphenylamine (Turian, 1950; Turian and Haxo, 1952; Jensen et al., 1958) demonstrated a net accumulation of saturated carotenoids. In some cases, it was possible to demonstrate a stoichiometric relationship between the accumulated saturated carotenoids and the more unsaturated ones upon removal of the inhibitor. Consequently, in many cases there exists a possibility for the conversion of the hydrogenated carotenoids to those which are less saturated. The results of ionone or heptenone treatment of *Rhodotorula* 48-23T are similar to those reported for treatment of cultures with similar inhibitors in other organisms (i.e., the accumulation of more saturated, alicyclic carotenoids). The proposed sequential pathway (Fig. 2) is, therefore, consistent with previous findings (Jensen et al., 1958; Porter and Anderson, 1962), structural relationships, results which have been reported using inhibitors to block synthetic carotenoid synthesis, and data derived from the analysis of carotenoids of 62-506 at various times during its growth.

An aldehydic pigment is postulated as an intermediate in the formation of torularhodin from torulin, although the pigment was not isolated in these experiments. Its inclusion is based upon its synthesis by Isler et al. (1959).

The development of previously unobserved carotenoids in *Rhodotorula* 48-23T after the treatment with inhibitors indicates that pathways for their formation are available in the organism, and would cast some doubt on the conclusions of Villoutreix (1960), that all carotenoids which an

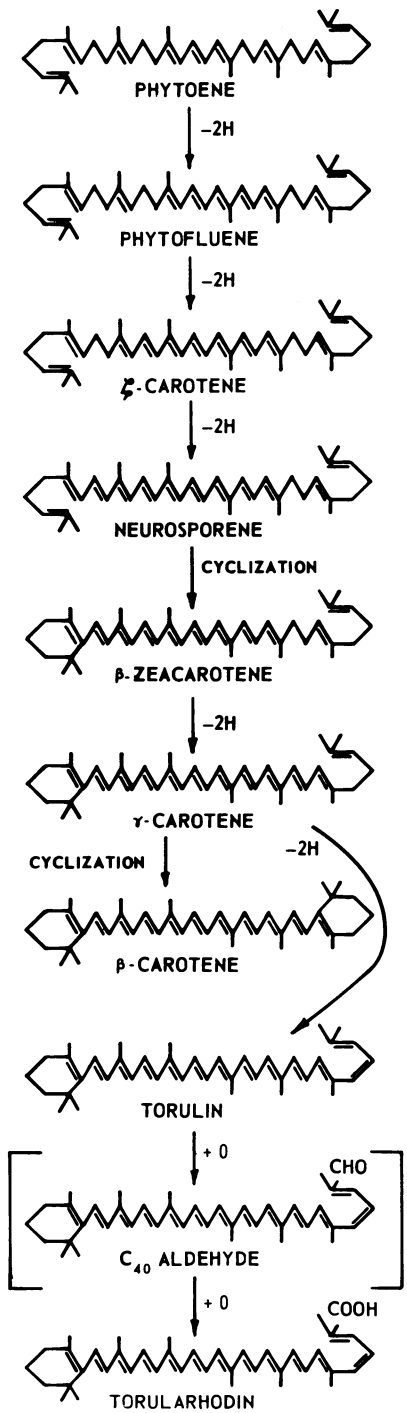

FIG. 2. *Proposed pathway for biosynthesis of yeast carotenoids.*

organism can form must be isolable from the untreated organisms by the extraction of large samples.

The occurrence of β-zeacarotene and β_1-zeacarotene in yeast has not previously been reported. The structure of β_1-zeacarotene is not known, but it is related to that of β-zeacarotene (Petzold, Quackenbush, and McQuistan, 1959). In all cases where these could be separated, the amount of β-zeacarotene isolated was greater than that of β_1-zeacarotene. Only β_1-zeacarotene was isolated from cultures grown on the rotary shaker.

Lycopene was not detected in any of the cultures, although the various treatments caused the level of neurosporene and β-zeacarotene to increase. These results do not rule out lycopene as an intermediate, but it would appear that at least the main pathway between neurosporene and γ-carotene is mediated by β-zeacarotene.

No xanthophylls containing a secondary hydroxyl group were found in this study. To our knowledge, no carotenoids containing this grouping have been reported in yeast, although they are extremely common in plant tissues.

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LITERATURE CITED

BONNER, J., A. SANDOVAL, T. W. TUNG, AND L. ZECHMEISTER. 1946. Changes in polyene synthesis induced by mutation in a red yeast (*Rhodotorula rubra*). Arch. Biochem. **10**:113–123.

CHICHESTER, C. O., P. S. WONG, AND G. MACKINNEY. 1954. On the biosynthesis of carotenoids. Plant Physiol. **29**:238–241.

CLAES, H. 1954. Analyse der biochemischen Syntheskette für Carotinoide mit Hilfe von *Chlorella*-Mutaten. Z. Naturforsch. **96**:461–470.

CLAES, H. 1956. Biosynthese von Carotinoiden bei *Chlorella*. 2. Mitt.: Tetrahydrolycopin und hycopin. Z. Naturforsch. **116**:260–266.

CLAES, H. 1958. Biosynthese von Carotinoiden bei *Chlorella*. Iv. Die Carotinsynthese einer *Chlorella*-Mutante bei anaerober Beichtung. Z. Naturforsch. **136**:222–224.

GOODWIN, T. W. 1956. Studies in carotenogenesis.

19. A survey of the polyenes in a number of ripe berries. Biochem. J. **62**:346–352.

GRAFF, M. M., R. T. O'CONNOR, AND E. L. SKAN. 1944. Purification of solvents for absorption spectroscopy. Ind. Eng. Chem. Anal. Ed. **16**:556–557.

ISLER, O., W. GUEX, R. RÜEGG, G. RYSER, G. SAUCY, U. SCHWIETER, M. WALTER, AND A. WINTERSTEIN. 1959. Synthesen in der Carotinoid-Reihe. Carotinoide vom Typus des Torularhodins. Helv. Chim. Acta **42**:864–871.

JENSEN, S. L., G. COHEN-BAZIRE, T. O. M. NAKAYAMA, AND R. Y. STANIER. 1958. The path of carotenoid synthesis in a photosynthetic bacterium. Biochim. Biophys. Acta **29**:477–498.

JENSEN, S. L., G. COHEN-BAZIRE, AND R. Y. STANIER. 1961. Biosynthesis of carotenoids in purple bacteria: a re-evaluation based on considerations of chemical structure. Nature **192**:1168–1172.

KARRER, P., AND E. JUCKER. 1950. The carotenoids. Elsevier Publishing Co., Inc., New York.

KAYSER, F., AND J. VILLOUTREIX. 1961. Biosynthèse des Caroténoides par *Rhodotorula mucilaginosa*. Etude d'un mutant obtenu par action des rayons X. Compt. Rend. Soc. Biol. **155**:1094–1095.

KOE, B. K., AND L. ZECHMEISTER. 1952. In vitro conversion of phytofluene and phytoene into carotenoid pigments. Arch. Biochem. Biophys. **41**:236–238.

MACKINNEY, G. M., T. NAKAYAMA, C. D. BUSS, AND C. O. CHICHESTER. 1952. Carotenoid production in *Phycomyces*. J. Am. Chem. Soc. **74**:3456–3457.

NAKAYAMA, T. O. M. 1958. The carotenoids of *Rhodopseudomonas* I & II. Arch. Biochem. Biophys. **75**:352–360.

NAKAYAMA, T., C. O. CHICHESTER, A. LUKTON, AND G. MACKINNEY. 1957. Phytoene production in *Phycomyces*. Arch. Biochem. Biophys. **66**:310–315.

NAKAYAMA, T., G. MACKINNEY, AND H. J. PHAFF. 1954. Carotenoids in asporogenous yeasts. Antonie van Leeuwenhoek J. Microbiol. Serol. **20**:217–228.

NASH, H. A., F. W. QUACKENBUSH, AND J. W. PORTER. 1948. Studies on the structure of ζ-carotene. J. Am. Chem. Soc. **70**:3613–3615.

PETZOLD, E. N., F. W. QUACKENBUSH, AND M. MCQUISTAN. 1959. Zeacarotene, new provitamins A from corn. Arch. Biochem. Biophys. **82**:117–124.

PORTER, J. W., AND D. G. ANDERSON. 1962. The biosynthesis of carotenes. Arch. Biochem. Biophys. **97**:520–528.

PORTER, J. W., AND R. E. LINCOLN. 1950. I. *Lycopersicon* selections containing a high content of carotene and colorless polyenes. II. The mechanism of carotene biosynthesis. Arch. Biochem. Biophys. **27**:390–403.

RABOURN, W. J., AND F. W. QUACKENBUSH. 1953. The occurrence of phytoene in various plant materials. Arch. Biochem. Biophys. **44**:159–164.

REYES, P. 1963. The influence of beta-ionone on carotenoid and ergosterol biosynthesis: its mechanism and metabolic site of action. Ph.D. Thesis. University of California, Davis, California.

RÜEGG, U. SCHWIETER, G. RYSER, P. SCHUDEL, AND O. ISLER. 1961*a*. Synthesen in der Carotinoid-Reihe. γ-Carotin sowie d,l-a-und β-Carotin aus Dehydro-β-apo-12'-carotinal (C_{25}). Helv. Chim. Acta **44**:985–993.

RÜEGG, R., U. SCHWIETER, G. RYSER, P. SCHUDEL, AND O. ISLER. 1961*b*. Synthesen in der Carotinoid-Reihe. Synthese von 7',8'-Dihydro-γ-carotin (β-Zeacarotin) und 3',4'-Dehydro-γ-carotin (Torulin). Helv. Chim. Acta **44**:994–999.

SIMPSON, K. L., T. O. M. NAKAYAMA, AND C. O. CHICHESTER. 1964. The biosynthetic origin of the carboxyl oxygen atoms of the carotenoid pigment torularhodin. Biochem. J. **92**:508–511.

SIMPSON, K. L., A. W. WILSON, E. BURTON, T. O. M. NAKAYAMA, AND C. O. CHICHESTER. 1963. Modified French press for the disruption of microorganisms. J. Bacteriol. **86**:1126–1127.

TURIAN, G. 1950. Recherches sur la biosynthèse des carotenoïdes chez un Bacille para-tuberculeux. III. Inhibition de la pigmentation par la diphénylamine. Helv. Chim. Acta **33**:1988–1993.

TURIAN, G., AND F. HAXO. 1952. Further use of diphenylamine for the study of carotenoid biosynthesis in Mycobacterium phlei. J. Bacteriol. **63**:690–691.

VILLOUTREIX, J. 1960. Les Caroténoides de *Rhodotorula mucilaginosa*. Etude de leur Biosynthèse a l'Aide de l'Analyse de Mutants et de l'emploi d'un Inhibiteur de la Caroténogénèse. Biochim. Biophys. Acta **40**:442–457.